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Chromosome First Aid

Minireview

Carol W. Greider
Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

Most people take the stability of chromosomes for granted. Some may imagine metaphase chromosomes lined up like a butterfly collection on a page, reassuringly in order and unchanging. However, evidence that has been accumulating for over half a century indicates that genome stability is a dynamic, not a static, process. In the 1940s natural chromosomes ends, or telomeres, were shown to be specialized structures inherently different from broken chromosome ends. McClintock demonstrated in maize that telomeres are required for the stable maintenance of linear chromosomes. Random ends generated by breakage fuse to generate dicentric chromosomes that undergo repeated cycles of breakage and fusion. Occasionally, during this cycle of breakage–fusion–bridge, chromosomes would apparently heal; their characteristic stickiness would disappear, and they would be well behaved in subsequent divisions (reviewed in Blackburn and Szostak, 1984).

In addition to sporadic healing events, McClintock observed developmentally regulated, tissue-specific chromosome healing. She found that the fate of a single chromatid broken at meiosis depends on both the tissue and the time in the cell cycle at which the broken end is present. When a single broken chromatid is generated at meiosis, rounds of breakage–fusion–bridge occur in the following mitotic divisions. After fertilization, the breakage cycle continues in the endosperm tissue, while in the zygotic tissue the broken end is healed. The healed chromosome is stably propagated throughout subsequent plant development. These observations suggested that an activity that heals broken ends is present in zygotic tissue but absent in the endosperm (McClintock, 1939, 1942).

In the past ten years there has been an explosive increase in our understanding of the molecular structure and function of telomeres. However, only very recently has the process of chromosome healing, initially documented over 50 years ago, been understood at the molecular level.

Telomere Structure and Synthesis

Telomeric sequences, first characterized in ciliates, are remarkably conserved throughout evolution. Functional telomeres consist of many tandem repeats of simple G-rich sequences. In Tetrahymena there are approximately 70 (TTGGGG) repeats, while in humans there are more than 600 (TTAGGG) repeats found at all chromosome ends. The number of repeats on any given chromosome varies, giving telomeric restriction fragments a characteristic fuzzy appearance on Southern blots. Because most DNA polymerases are not able to replicate completely the molecular end of a DNA molecule, the mechanism of telomere replication was the topic of speculation for many years (reviewed in Blackburn and Szostak, 1984). However, the discovery of the enzyme telomerase in Tetrahymena presented a simple explanation for both the structure and replication of telomeres. Tetrahymena telomerase synthesizes telomeric (TTGGGG) repeats de novo onto chromosome ends. Thus, shortening due to incomplete replication is balanced by net lengthening. Telomerase enzymes have been characterized in three ciliate species and in human cells. Telomerase is a very unusual DNA polymerase, containing both essential protein and RNA subunits. The small RNA component provides the template for the telomere repeats that the enzyme synthesizes (reviewed in Blackburn, 1991).

Since the early observation of chromosome breakage and healing in plants, numerous examples of chromosome healing have been described. Most can be roughly divided into two categories of healing events: developmentally programmed chromosome fragmentation and sporadic healing of accidentally broken chromosomes.

Developmentally Programmed Healing

The term chromatin diminution has been used to describe the fragmentation of chromosomes and the elimination of DNA during differentiation of somatic lineages from the germline. The term was originally used by Boveri to describe the loss of heterochromatin in somatic cells in Parascaris (reviewed in Pimpinelli and Goday, 1989; Tobler, 1986). A similar process has been well documented in ciliated protozoa. Ciliates are unicellular organisms, yet each cell contains two different types of nuclei, the germline micronucleus and the somatic macronucleus. After conjugation, a mitotic product of the zygotic nucleus undergoes macronuclear development involving chromosome fragmentation, DNA elimination, and DNA amplification. Chromosome fragmentation has been characterized at the molecular level in the ciliates Tetrahymena, Oxytricha, and Euplotes (reviewed in Yao, 1989). During fragmentation, telomeric sequences are added onto the newly generated macronuclear DNA ends. When the sequence of cloned macronuclear telomeres is compared with cloned micronuclear sequences, no G-rich telomeric repeats are found in the micronuclear precursors.

Telomere addition in ciliates appears to be sequence independent. No consensus sequence for telomere addition sites has been found in the numerous examples studied. In Tetrahymena, a consensus sequence for chromosome breakage is found in micronuclear-limited DNA (reviewed in Yao, 1989). The exact amount of DNA lost around the breakage site and the exact site of telomere addition differ with each independent development event. This suggests that after sequence-directed cleavage, a competition may arise between exonucleolytic degradation of DNA and telomere addition.

A dramatic example of this somewhat random process of telomere addition comes from Paramecium mutants in which chromosome fragmentation is aberrant. These mutations result in fragmentation in the middle of the coding region for a surface antigen gene. Eight different macronuclear telomere addition sites were compared with the wild-type antigen locus. The data suggest that telomere addition can occur onto any sequence (Forney and Black-

burn, 1988; reviewed in Blackburn, 1991). Further evidence from Paramecium indicates that there is little or no sequence specificity for sites of telomere addition. Microinjection of linear DNA fragments into the macronucleus results in both the addition of telomeres and the replication of the injected DNA. Even small pBR322 restriction fragments are crowned with telomeres (Gilley et al., 1988).

Molecular studies of chromatin diminution in Ascaris have revealed a very similar story to that found in ciliates (Müller et al., 1991). In Ascaris the germline and somatic cell lineages are differentiated early in development. In somatic lineages, repetitive DNA is eliminated, the chromosomes are reduced in size, and new telomeres are generated (reviewed in Tobler, 1986). When germline sequences were compared with the corresponding cloned somatic telomeres, it was apparent that de novo telomere addition must have occurred. A broad chromosomal breakage region was identified in which new telomeres appear to be added at random locations. No consensus sequence could be identified for telomere addition (Müller et al., 1991).

Spontaneous Chromosome Healing

In addition to developmentally programmed chromosome fragmentation that occurs in a few organisms, spontaneous healing has been documented in a variety of eukaryotes. Healing events seem to come in two flavors, recombination-mediated telomere acquisition and de novo telomere synthesis. In yeast, healing by recombination is the most likely fate for a broken end. Dicentric chromosomes broken at mitosis most often heal by homologous recombination. Gene conversion with a sister chromatid, homolog, or subtelomeric repetitive DNA allows the entire end of a chromosome to be duplicated. These conversion events result in the transfer of sequences internal to the simple telomere repeats onto the healed end (Dunn et al., 1985; Haber and Thorburn, 1984; Jager and Philippsen, 1989). Healing of unstable broken ends also has been reported in Drosophila. Although the mechanism of healing is not fully understood, the evidence suggests that it, too, is a recombination-mediated event (Biessmann et al., 1990).

In addition to recombinational telomere acquisition, examples exist in yeast in which no transfer of subtelomeric sequence was found, suggesting that healing occurred by de novo telomere addition (Haber and Thorburn, 1984; Murray et al., 1988; Jager and Philippsen, 1989). The presence of Tetrahymena telomeric sequences at or near the ends of linear DNA stimulates the addition of yeast telomeric sequences and allows plasmid maintenance. Several examples were found in which the new telomeric DNA was added onto pBR322 sequences up to 100 bp away from the Tetrahymena TTGGGG repeats. Although the presence of the Tetrahymena sequence increased the efficiency, healing appears to have occurred by de novo addition (Murray et al., 1988).

De novo addition of telomere sequences has also been implicated in healing in Schizosaccharomyces pombe, Plasmodium, and humans. In Plasmodium and humans, the healed chromosomes have been characterized at the sequence level. In both cases there is an abrupt transition from the coding sequence of a known gene to telomeric repeats. The absence of any intervening unrelated sequence suggests that telomeres were added de novo onto a free broken end (reviewed in Blackburn, 1991).

Telomerase Can Mediate Healing In Vitro

The ability of telomerase to synthesize telomeric sequence de novo make it a prime candidate for the activity that heals chromosomes in vivo. However, in vitro telomerase shows a high degree of specificity for telomeric sequence primers over random sequence primers. This specificity raises the question of whether telomerase can add telomeric repeats onto random chromosome breakage sites. Recent in vitro evidence indicates that telomerase can add telomere repeats onto nontelomeric sequences. Tetrahymena telomerase will elongate chimeric oligonucleotides that have 12 bases of TTGGGG at the 5′ end and up to 36 nucleotides of nontelomeric sequence at the 3′ end. Thus, hybridization of the 3′ end of primers to the telomerase RNA is not required for repeat addition (Harrington and Greider, 1991). This requirement for a telomeric sequence near but not at the site of telomere addition is similar to that found during de novo healing of yeast chromosomes in vivo (Murray et al., 1988).

Similar experiments with human telomerase indicate that this enzyme is also capable of carrying out chromosome healing. Human telomerase will elongate oligonucleotides corresponding to a site of chromosome breakage and healing associated with α thalassemia (Morin, 1991). These studies provide biochemical evidence that telomerase is capable of chromosome healing by de novo addition of telomere sequences onto nontelomeric DNA.

Telomerase Does Mediate Healing In Vivo

The studies cited above suggest how telomerase could mediate healing. However, all the primers used either contain some telomeric sequence or are G-rich and therefore telomere-like. De novo telomere formation in Tetrahymena occurs in AT-rich sequences with no nearby telomere repeats. Although telomerase will not elongate primers with this sequence in vitro (Spangler and Blackburn, 1988), Yu and Blackburn (1991) now demonstrate that it is responsible for de novo telomere synthesis at AT-rich sites in Tetrahymena. The introduction of a mutation into the RNA template region of telomerase was used to mark it in vivo. Tetrahymena cells containing the mutant telomerase were then mated and allowed to undergo macronuclear development. Telomeres cloned from the progeny cells contain the mutant sequence as the first repeat added onto AT-rich macronuclear ends. Thus, telomerase must directly synthesize new telomeres during developmentally programmed chromosome healing (Yu and Blackburn, 1991).

In addition to identifying the perpetrator in healing, the experiments also identify two important properties of telomerase in vivo. First, the interspersion of mutant and wild-type repeats on the new ends suggests that telomerase may be distributive in vivo, although it is processive in vitro (Greider, 1991). Because the most internal telomere repeats do not seem to turn over during vegetative growth, the interspersed pattern probably reflects one synthesis event by telomerase. Another explanation for

the interspersed pattern could be that a single telomerase enzyme contains two RNA molecules, and the pattern observed was generated by the processive action of a bivalent enzyme. Second, the mutant RNA that specifies T_2G_5 repeats often generated T_2G_{5-8} sequences. These sequences were most likely created by slippage of the template on the elongating telomere. The synthesis of these irregular repeats suggests how telomerase in yeast or Dictyostelium might generate the TG_{1-3} and AG_{1-8} telomeres characteristic of these organisms (Yu and Blackburn, 1991).

Conclusion

Chromosome breakage and healing has been documented since the time it was first possible to follow and visualize chromosomes. How this process of stabilizing broken ends occurred was not clear. It is now certain that telomerase is responsible for new telomere synthesis in Tetrahymena and, by inference, it is probably also involved in de novo healing events. Our survival depends on the faithful transmission of intact chromosomes from one cell generation to the next. Most of the time this process occurs without error. However, when accidents do occur, it is nice to know that telomerase is on call to provide a molecular Band-aid for patching up the broken ends.

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